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Faculty of Natural Resources and Agricultural Sciences

Effects of soil substrate and nitrogen fertilizer on biomass production of *Acacia senegal* and *Acacia sieberiana* in North Eastern Uganda

Moses Otuba

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Effekter av marksubstrat och kvävegödsel på biomassaproduktion hos *Acacia senegal* och *Acacia sieberiana* i nordöstra Uganda

Moses Otuba

Supervisor:
Professor Dr. Martin Weih, Swedish University of Agricultural Sciences, Department of Crop Production Ecology

Examiner:
Professor Dr. Lars Andersson, Swedish University of Agricultural Sciences, Department of Crop Production Ecology

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Dedication

To my family and friends.

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Moses Otuba

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List of Acronyms and Abbreviations

Ca: calcium

cf: compared with

ICRAF: International Centre for Research in Agroforestry

K: potassium

Mg: magnesium

MUK: Makerere University , Kampala

N: nitrogen

NARO: National Agricultural Research Organisation

NaZardi: Nabiun Zonal Agricultural Research Institute

NaSARRI: National Semi Arid Resources Research Institute

NEMA: National Environment Management Authority

NFA: National Forestry authority

n.d : no date

P: phosphorus

S.I: Swedish Insitute

SLU: Swedish University of Agricultural Sciences

USA: United States of America

Abstract

A study to determine the effects of soil substrate and nitrogen fertilizer on the growth rate and biomass production of *Acacia senegal* and *A. sieberiana* in North Eastern Uganda was conducted between February and June, 2012**.** The objectives of the study were to determine the effects of soil substrate and nitrogen fertilizers on the growth rate and biomass allocation below-ground and above-ground of *A. senegal* and *A. sieberiana* seedlings. A multi-factorial experiment design was set up for collecting the data to address the two objectives of this study. There was a significant effect ($P \leq 0.05$) of soil substrate and the species- soil interaction on growth rate between two acacia species. The mean relative leaf length of *A. sieberiana* (0.013 mm mm^{-1} d^{-1}) in unfertilized (N0) soil A was higher compared to those in the soils treated with N fertilizer. Analysis of variance shows that while there was no significant effect ($P \leq 0.$ 05) of species, soil substrate and species- soil interaction on the relative stem and leaf biomass growth, relative root biomass growth, and the root biomass allocation and stem and leaf biomass allocation at final harvest, there was a significant species effect ($P \leq 0.$ 05) on leaf N concentration *A. senegal* seedlings had a higher stem and leaf biomass allocation (83%) in soil substrate A with N0 compared to the N treatments. *A. sieberiana* and *A. senegal* planted in the unfertilized soil B produced higher relative stem and leaf biomass growth (0.034 g g^{-1} d^{-1}) compared to those treated with N fertilizer. *A. senegal* had a higher leaf N concentration (7.1%, 6.6%, and 6.3%) in soil B treated with N50, N100 and N150 mg / plant, respectively, compared to the N0 treatments. Analysis of Pearson correlation showed that there was no statistically significant effect ($P \leq 0.$ 05) of the leaf N concentration on the growth traits of the two acacia species in all treatments. It can be concluded that unfertilised soil substrates (N0) supported higher growth rate of both *A. senegal* and *A. sieberiana* seedlings than fertilization with a pure ammonium nitrate solution lacking other nutrients important for growth. It is recommended that further investigations using a complete and a balanced nutrient solution with small quantities of N fertilizers less than the rates used in this study be carried out. Application of the N treatment produced less stems and leaves biomass allocation compared to N0 treatments. Therefore, N fertilizers may not be used to enhance biomass production of two acacia species at the age 2 months. Further research can be conducted on the effects of N fertilizer on two acacia species for a long period of experiment. The two soil substrates affected the growth traits of the two acacia species differently while that on biomass allocation in the same way. The leaf N concentration did not enhance the growth traits of the two acacia species.

Key Words: Acacia senegal, *Acacia sieberiana*, nitrogen, fertilizer, growth rate, biomass allocation, soil substrate.

1.0 Introduction

1.1 Background

Acacia tree resources are estimated to be 1250 species in the world, 134 species of which are native to Africa and the other remaining species are found in Australia, Asia and America (Wickens *et al*., 1995). The acacia species are xerophytes, i. e. plants which grow in the arid or semi-arid areas, which cover 55 % of the land surface in Africa. Acacia species are widely distributed in the drier parts of tropical Africa, from Senegal and Mauritania in the west to Eritrea and Ethiopia in the North-East and to South Africa (Wekesa *et al*., 2010).

The most dominant species in Africa are Sudan gum arabic, *Acacia senegal (*L*)* and Paper bark thorn*, Acacia sieberiana (De Wild)* (Serif el Din, 1991; Wickens *et al*., 1995). The trees grow well on dry and rocky hills, and in low-lying dry savannas with annual rainfall of approximately 250 - 350 mm. They also tolerate a maximum temperature of 50 ^{o}C and a minimum temperature close to 0 ^{o}C (ICRAF, n.d) and soil pH of approximately between 5.0 and 8.0 (Bekele-Tesemma *et al.,* 1993; Cheema and Qadir, 1973).

Despite the unfavourable growth conditions for most plants in the arid and semi-arid areas, Acacia species are appreciated in these areas because of their specific morphological and physiological attributes enabling them to cope with those conditions. The trees are used by the rural communities and manufacturing industries in many ways, for example *A. senegal* to restore soil fertility in rain-fed sorghum-producing areas consisting of clay soils in the Blue Nile region, Sudan (Raddad *et al*., 2006). As cited by Raddad *et al.,* (2006), El Houri (1986) points out that the trees can be used for rotational bush–fallow systems. The rotational system consists of relatively short periods of crop cultivation followed by longer periods of fallow under mainly naturally regenerated *A. senegal* trees when soil fertility declines.

A. senegal and *A. sieberiana* are also tapped for gum exudates when they reach the age of 15 – 20 years growing on either the bushes or cultivated land. The gum normally is classified in two qualities, thus higher and lower grades. The higher grade of gum is used to inhibit sugar crystallization in confectionary products, and as emulsifier in the production of soft drinks (Osman *et al*., 1993a; Baldwin *et al.,* 1999). It is also used as an adhesive to clarify wine (Anderson & De Pinto, 1980; Baldwin *et al.,* 1999) and to encapsulate pharmaceuticals (Joseleau & Ullmann, 1990; Baldwin *et al.,* 1999). The lower grades of gum are used in non-food related industries, for example printing and textiles and in the production of explosives (Anderson & De Pinto, 1980; Baldwin *et al.,* 1999).

The gum exudates tapped from the two acacia species also contribute to environmental rehabilitation and desertification control through stabilization, reduction of surface run-offs and sheet erosion and soil micro-climate improvement in the Kordofan region of Sudan (Egadu *et al.,* 2007). In addition, the trees are used as fencing materials, fuel wood, poles, fibre, crafts, medicine and tannins in Uganda.

Following the major role of *A. senegal* and *A. sieberiana* in the development of local communities in Uganda specifically and globally in general, there are plans to invest into these two species with the aim to increase biomass yield and the production of gum exudates (Egadu *et al*., 2007). Increased use of the two species could be an opportunity of alleviating poverty in the rural areas particularly in the dry zones. However, little is known about the influence of soil characteristics and plant nutrients, especially nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) on the growth rate and biomass production of these two species under the operational nursery conditions in Uganda.

Recent studies show that productivity and fertility of soils in the world are declining due to degradation and intensive use of soils without a consideration of proper soil-management practices (Gruhn *et al*., 2000; Cakmak, 2002). Inadequate and unbalanced supply of mineral nutrients and impaired soil fertility are particular problems, causing decrease in global food production, especially in the developing countries.

It is estimated that around 60 % of cultivated soils have growth-limiting problems associated with mineral nutrient deficiencies and toxicities (Cakmak, 2002). Byrnes and Bumb (1998) state that fertilizer consumption will increase by around 2-fold to achieve the needed increases in food production in the next 20 years. It seems that in the coming decades, plant-nutrition-related research will be a high- priority research area contributing to crop production and sustaining soil fertility.

Nitrogen, P, and K play an important role in limiting plant growth and crop yield (Evans, 2000). Nitrogen is primarily required for increasing plant growth and crop yield more than any other nutrients. The absence of N in the plants is often associated with slow growth, reduced leaf size, yellowing; short branches, premature fall colour and leaf drop, and increase the likelihood of some diseases. Natural ecosystems respond to nitrogen fertilisation with increased productivity or change in species composition (Lee *et al.,* 1983; Field, 1986), despite the presence of nitrogen in soils. Much of the N in soils is stored within the soil humus in forms that plants cannot easily access (Galloway *et al*., 2004).

High amounts of nutrients often result in excessive shoot and foliage growth, reduced root growth, reduced fruit quality, low plant food reserves, and increased susceptibility to environmental stresses and some plant diseases (Evans, 2000). Excess N can also lead to an accumulation of nitrate in the edible foliage of plants such as spinach and forage crops. Ingestion of such high-nitrate foods can pose possible health risks to animals and humans.

The availability of adequate amounts of P in the soil increases the rate of photosynthesis, respiration, energy storage and transfer, cell division, and cell enlargement in plants. It also promotes early root formation and growth, and the production of flowers, fruits, and seeds (Barrow, 1983). These processes occur when soil is enriched with phosphorus fertilizer as it is often present in unavailable forms or in forms that are only available outside of the rhizosphere (Schachtman *et al.,* 1998).

In many agricultural soils in which P has been applied to ensure plant productivity, the recovery of P by crop plants in a growing season is very low, because more than 80 % of the phosphorus becomes immobile and unavailable for plant uptake as result of adsorption, precipitation, or conversion to the organic form (Holford, 1997).

Besides the importance of N and P in plant growth, fertilising plants with K is also vital in many physiological processes such as improving the rate of photosynthesis, translocation of photosynthates into sink organs, maintenance of turgescence, activation of enzymes, and reducing excess uptake of ions in saline and flooded soils (Marschner and Marschner, 1995; Mengel and Kirkby, 2001). Thus, K contributes to the survival of crop plants under environmental stress conditions, increases disease resistance, and improves winter hardiness (Cakmak, 2002). The use of fertilizers remains an effective means of rapidly increasing acacia tree cover and other dominant tropical trees (Field, 1986).

Planting tree seedlings on disturbed tropical sites can accelerate forest recovery, but the success is usually hampered by several factors. The retrieval of subsoil nutrients by the trees, for instance, is generally greatest with a deep rooting system and a high demand for nutrients, water and/or nutrient stress occurs in the surface soils and considerable reserves of plant-available nutrients or weatherable minerals exist in the subsoil (Buresh, 1995; Buresh and Tian, 1997).

Greater capture of subsoil resources by roots would be expected for water and mobile nutrients such as nitrate than for less mobile nutrients such as phosphorus. There is normally little potential of trees to capture phosphorus from below the rooting depth of crops because the plant-extractable phosphorus is normally low in the sub-soil (Breman and Kessler, 1995).

The optimum nutrient concentration required for the maximum growth also varies between parts of a tree and between the species and even the provenances (Savill *et al.,* 1997). The optimum concentrations also differ according to which growth parameter is considered. The optimum for the growth in height is often at a lower concentration than the growth in volume. Concentrations also vary significantly with plant size or age of plant (Miller *et al*., 1981; Miller 1984).

To promote the production of *A. senegal* and *A. sieberiana* for high yields of biomass and improved growth rate in Uganda, more knowledge is needed on the nutrient requirements of these two species, to equip the stakeholders with relevant information for improved silvicultural management practices under the nursery operations. Information on the plant nutrient content would help the tree growers to understand how different soil substrates and nitrogen fertilizer affect the growth rate and biomass yield of *A. senegal* and *A. sieberiana*. The findings can also be used by other interested institutions for example National Forestry Authority (NFA), National Environment Management Authority (NEMA) and other research institutions to develop appropriate the policies for enhancing *A. senegal* and *A. sieberiana* growing projects and programmes in the region.

1.1 Objectives

i) To determine the effects of soil substrates and nitrogen fertilizers on the growth rate of *A. senegal* and *A. sieberiana* seedlings.

ii) To determine the effects of soil substrates and nitrogen fertilizers on biomass allocation below-ground and above-ground of A. *senegal* and *A. sieberiana* seedlings.

1.2 Hypotheses

i) The magnitude of effect of soil substrates on the growth rate, biomass allocation and leaf nitrogen concentration varies between the two acacia species.

ii) The leaf N concentration is functionally related to the growth traits of the two acacia species.

2.0 Materials and Methods

2.1 Plant Material and Substrate

Seeds of *A. senegal* and *A. sieberiana* and soil samples were collected from Moroto and Kotido districts in North Eastern Uganda, East Africa (Figure 2.1). The region is located between latitude 1° 30'– 4° N, longitude 33° 30'– 35° E at an altitude; of 1400 m above sea level (Grade *et al*., 2009). The National Environment Management Authority, reports Moroto and Kotido districts in Karamoja region as semi-arid with distinct wet and dry seasons (NEMA 1997a, b; Egadu *et al*., 2007).

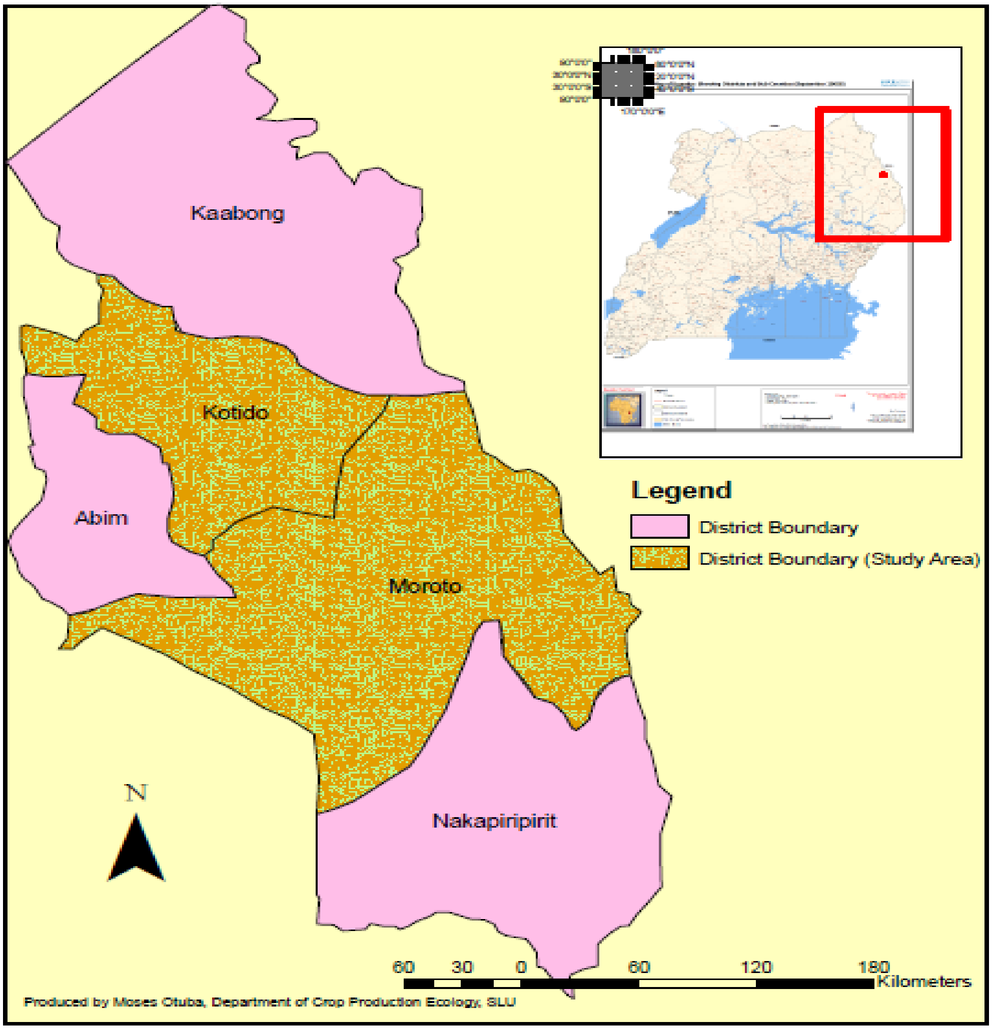


Figure 2. 1. Location of Kotido and Moroto districts in Uganda..

The amount of rainfall is inadequate, not evenly distributed and unreliable (Wilson & Rowland, 2001; Department of Meteorology Uganda, 2002; Egadu *et al*., 2007). The mean annual rainfall is about 600 mm per year with the higher amount in the surrounding mountain ranges. The average annual temperatures range from 30 ºC to 35 ºC (Grade *et al*., 2009). The soils are classified as vertisol and some are sandy easy to dig while others are heavy difficult to dig. The vegetation has faced gradual degradation since the 1960s because of deforestation, overgrazing, fires and mono cropping.

2.2 Experimental Design

The seeds of the two acacia species and soil (0 - 25 cm) were collected randomly from a sample plot measuring 30 m x 30 m in each district and a multi-factorial experiment was set up. Soil A and B from Moroto and Kotido district, respectively, were air dried and sieved (2 mm) to remove large debris like leaves and stones. Soil A was lighter than soil B, i.e it was easier to excavate soil sample A than soil sample B. However, both of them were crushed into small particles (Figure 2.2), then mixed separately and filled in 160 larger U- plastic pots; of 2 L volume for establishing the experiments in the greenhouse.

Figure 2.2. Soil A and B crushed into small particles

Before sowing the seeds into the larger plastic pots, they were scarified with concentrated sulphuric acid, H_2SO_4 for 20-30 min to break the dormancy and then rinsed several times with tap water. The seeds were sown 2-3 per pot to reduce risks of gap filling, topped with sand to maintain equal volume and then watered daily throughout the entire experiment duration of 8 weeks. To avoid nutrient leaching, the pots were placed on plastic polythene sheet. After 1-2 weeks from the start of experiment, seedling number per pot was reduced, leaving only a single healthy seedling per pot.

A total of 160 seedlings were raised in the nursery shade for one month, of which 80 seedlings of *A. senegal,* 40 each were grown in soil A and B, respectively, and another 80 seedlings of *A. sieberiana,* 40 each grown in soil A and B.

Prior to the start of ammonium nitrate (NH_4NO_3) treatments, 32 seedlings were harvested, i.e. 8 replicate seedlings for each soil type (2) and species (2) to determine initial shoot height, leaf length, shoot and root biomass (i.e. biomass allocation), as well as leaf N content. During the experiment, the seedlings were grown in the greenhouse with humidity of 78 % and temperature of 30 ºC at National Semi Arid Resources Research Institute (NaSARRI) in Uganda. The seedlings were subjected to four rates of N treatments (0, 50, 100, 150 mg per plant) as a single dose of ammonium nitrate at the start of experiment and using a randomized complete block design. Eight replicate seedlings were grown in each species (2), soil type (2) and N fertilizer treatment (4) combination for one month.

At the end of the N fertilisation experiment, the remaining 128 seedlings were harvested to determine the final shoot height, leaf length, shoot and root biomass (i.e. biomass allocation), as well as leaf N content. The seedlings were also irrigated with deionised water to reduce the concentration of ammonium nitrate immediately after its exposure to seedlings.

2.3 Measurements

The shoot heights and leaf length of the two acacia species in the soil A and B substrates were measured before and after treatment of the seedlings with N fertilizer. Seedling heights were measured from the soil surface in the pots to the end of the shoot while the length of three leaves at bottom of each plant were measured from axillary bud by use of a tape measure.

The harvested leaves, stems and roots were oven dried for 48 hours at 70 ºC to determine the initial and final dry weights at the Department of Agricultural Production, Makerere University, Kampala in Uganda. Leaf N concentration was determined from 80 acacia samples i.e 16 samples analysed after first phase of seedling harvesting and then 64 after second phase using a Kjeldahl method (Jones, 1991).

2.4 Statistical Data Analysis

The functional approach to plant growth analysis (Hunt, 1982; Hunt 1990) was used to compare seedling growth rates, biomass allocation in various species and treatment combinations, by means of relative growth rates (Kozlowski & Pallardy, 1997). Thus, the relative height growth RGR_{HT}, relative leaf length RGR_{LL}, relative stem and leaf biomass growth RGR_{SLB}, and relative root biomass RGR_{RB} were computed according to the following;

RGR_{HT} ($cm\ cm^{-1}\ d^{-1}$) = ($InHT_2 – InHT_1$) / (t_2-t_1)..1

RGR_{LL} ($mm\ mm^{-1}\ d^{-1}$) = ($InLL_2 – InLL_1$) / (t_2-t_1)...2

RGR_{SLB} ($g\ g^{-1}\ d^{-1}$) = ($InSLB_2$ - $InSLB_1$) / (t_2-t_1)..3

$RGR_{RB\ (}$ $g\ g^{-1}\ d^{-1}$) = (In RB_2 – $InRB_1$) / (t_2 -t_1)...4

where t_2 : final time (weeks), t_1 : initial time, HT_2 : final seedling height(cm), HT_1 : Initial seedling height(cm), LL_2 : final leaf length (mm), LL_1 : initial leaf length (mm), SLB_2 : final stem and leaf dry weight (g), SLB_1 :initial stem and leaf dry weight (g), RB_2 : final root dry weight (g), RB_1 : initial root dry weight (g).

The experiment was analysed by general linear models with the species and substrates (soil types) as the main factors using Microsoft Office Excel 2007. Two-way analysis of variance (ANOVA) was used to test if the magnitude of effect of soil substrates on growth rate, biomass allocation and leaf nitrogen concentration varies between the two acacia species. The least square means were used to detect significance of on growth rate, biomass allocation and leaf nitrogen concentration of the two acacia species. An effect was considered to be significant if its *p*-value was ≤0.05. On the other hand, Pearson correlation analysis was used to test whether the leaf N concentration functionally relates to the growth, i,e height and leaf length of the two acacia species. An effect was considered to be significant if its correlation coefficient was positive value and if its *p*-value was ≤0.05 .

3.0 Results

3.1 Growth rate

Analysis of variance (ANOVA) of data on growth rate showed that there was a significant effect ($P \leq 0.05$) of soil substrate on relative height growth (RGR_{HT}), as well as significant species (S) effect and species-soil (S x SN) interaction on relative leaf length (RGR_{LL}) between the two acacia species when grown without N fertilization (Table 1).

Table 1. Summary of analysis of variance (ANOVA) on the effects of species, soil substrate and their interaction on relative growth height (RGR_{HT}), relative leaf length (RGR_{LL}) of A.senegal and A. sieberiana grown in the N0 treatment (no N fertilization).

Source	SS	df	MS	F-value	P-value	LoS
RGR_{HT} (cm cm^{-1} d^{-1})						
Species (S)	3.511×10^{-6}	1	3.51×10^{-6}	0.1015	0.7524	N.S
Soil substrate (SS)	0.00016	1	0.00016	4.6311	0.0402	*
(S x SS)	1.326×10^{-5}	1	1.32×10^{-5}	0.3833	0.5408	N.S
Error	0.00097	28	3.46×10^{-5}			
RGR_{LL} (mm mm^{-1} d^{-1})						
Species (S)	0.00017	1	0.00017	6.0490	0.0204	*
Soil substrates (SS)	3.4×10^{-7}	1	3.4×10^{-7}	0.0121	0.9133	NS
(S x SS)	0.00027	1	0.00027	9.4772	0.0046	*
Error	0.0046	28	2.82×10^{-5}			

*LoS: Level of Significance, *: significant at $P \leq 0.05$, N.S ; Not Significant*

The RGH_{HT} of *A. sieberiana* and *A. senegal* were significantly higher in the N0 treatments compared to those of N50, N100 and N150 treatments. (Figure3.1a). While *A. siberiana* had the highest RGR_{HT} in soil B of the N50, N100 and N150 treatments, it had very low RGR_{HT} in soil A (Figure 3.1a).

The RGR_{LL} of *A. senegal* and *A. sieberiana* in this study varied depending on the soil substrates and levels of N treatments. The results show that RGR_{LL} of *A. sieberiana* (0.013 mm mm^{-1} d^{-1}) in unfertilized soil A (N0 treatments) was higher compared to those in the soils treated with N fertilizer (N50, N100 and N150) (Figure 3.1b). *A. senegal*, however, had higher RGR_{LL} (0.012 mm mm-^{1} d^{-1}) in soil B treated with N50 mg / plant compared to those treated in soil A and B with N100 and N150 mg / plant.

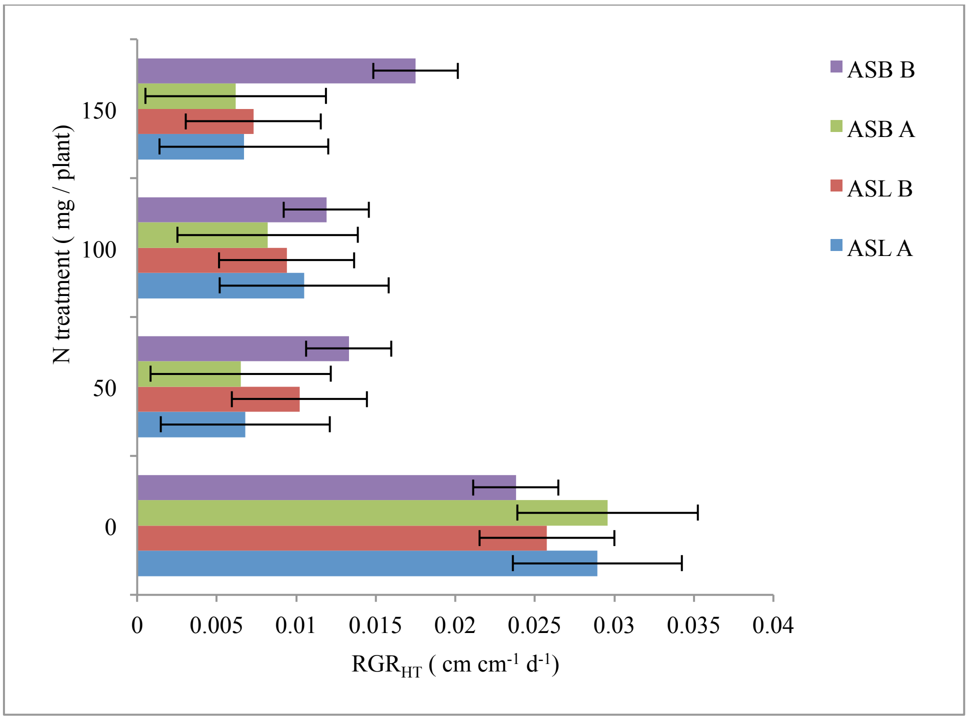


Figure 3.1a: The influence of nitrogen (N) fertilizer on mean relative growth height (RGR_{HT}) A. senegal (ASL) and A.sieberiana (ASB) seedlings in soil A and B. Bar errors show standard errors.

Figure 3.1b; A comparison of height of A.sieberiana seedlings in the pots treated with N fertilizer with those of N0 treatments in greenhouse. (From right to left; N0. N50, N100, and N150 mg / plant).

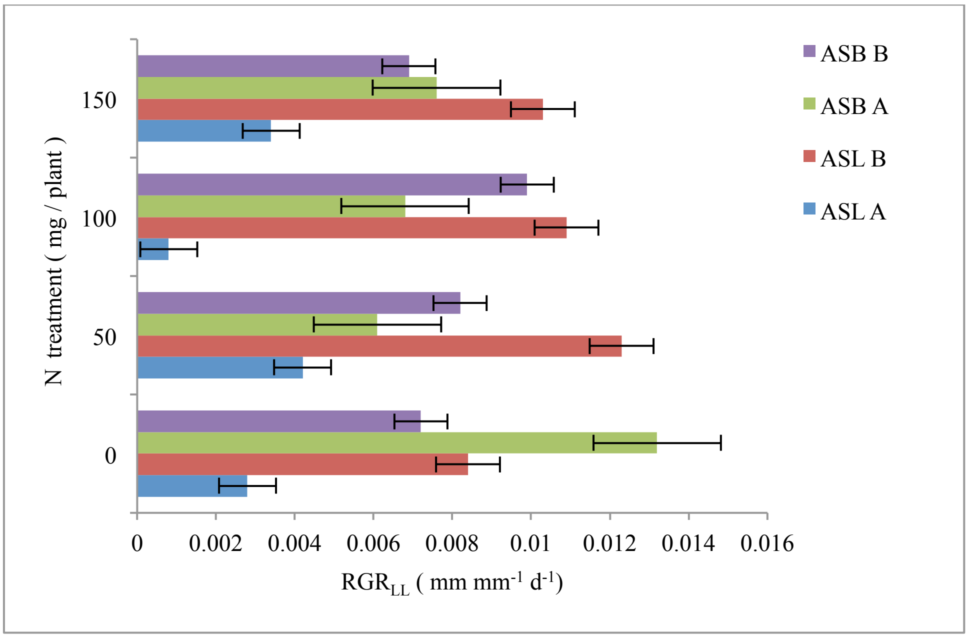


Figure 3.1c: The influence of nitrogen (N) fertilizer on mean relative leaf length (RGR_{LL}) of A.senegal (ASL) and A.sieberiana (ASB) seedlings in soil A and B. Bar errors show standard errors.

Figure 3.1 d. Acacia seedlings being treated with the N fertilizer in the greenhouse.

3.2 Biomass allocation above-ground and below-ground

Analysis of variance of data on the effects of species, soil substrate and their interaction had no significant impact ($P \leq 0.05$) on relative root biomass growth (RGR_{RB}), relative stem the leaf biomass growth (RGR_{SLB}) between the two acacia species. While there was also a highly significant effect ($P \leq 0.05$) of species and soil substrates on the leaf N concentration of the final harvest, those on the root biomass and stem and leaf biomass allocation did not show any significant effect between the two acacia species (Table 2).

Table 2. Summary of analysis of variance (ANOVA) on the effects of species, soil substrate and their interaction on relative stem and leaf biomass growth (RGR_{SLB}), relative root biomass growth (RGR_{RB}) and leaf nitrogen concentration of A.senegal and A. sieberiana grown in the N0 treatment (no N fertilization)

Source	SS	df	MS	F-value	P-value	LoS
RGR_{SLB} (g g^{-1} d^{-1})						
Species (S)	8.32 x10^{-5}	1	8.32 x10^{-5}	0.2829	0.5991	N.S
Soil substrates (SS)	4.9 x10^{-5}	1	4.9 x10-^{5}	0.1667	0..6862	N.S
(SX SS)	0.00090	1	0.0009	3.0707	0.0907	N.S
Error	0.00824	28	0.0003.			
RGR_{RB} (g g^{-1} d^{-1})						
Species (S)	0.00036	1	0.0004	0.6904	0..4313	N.S
Soil substrate (SS)	0.00208	1	0.0021	4.0037	0.0551	N.S
(S x SS)	0.00067	1	0.0007	1.2954	0.2647	N.S
Error	0.01454	28	0.0005			
Root biomass allocation (%)						
Species (S)	0.25936	1	0.2594	2.9443	0.097226	N.S
Soil substrates (SS)	0.33581	1	0.3358	3.8122	0.0609	N.S
(S x SS)	0.03617	1	0.0362	0.4107	0.5268	N.S
Error	2.4665	28	0.0881			
Stem and leaf biomass allocation (%)						
Species (S)	0.0295	1	0.0295	0.3883	0.5382	N.S
Soil substrates (SS)	0.0436	1	0.0436	0.5739	0.4551	N.S
(S x SS)	0.1014	1	0.1014	1.3353	0.2576	N.S
Error	2.1253	28	2.1253			
Leaf N concentration (%)						
Species (S)	14.1770	1	14.177	12.439	0.004	*
Soil substrate (SS)	0.2980	1	0.2830	0.2480	0.627	NS
(S x SS)	2.7920	1	2.7920	2.4490	0.144	NS
Error	13.6770	12	1.1400			

*LoS: Level of Significance, *: significant at $P \leq 0.05$, N.S; Not Significant*

There was more biomass allocated to the stems and leaves of *A. senegal* and *A. sieberiana* in soil substrates A and B compared to that of their root system in this study (Table 3). *A. senegal* seedlings had a higher stem and leaf biomass allocation (83%) in soil substrate A with N0 compared to those in N50, N100 and N150 mg / plant (N treatments). In soil B experiments treated with N150 mg / plant, *A. sieberiana* seedlings had equal biomass allocation (50%) for their stems and leaves and the roots (Table 3).

Table 3. Proportion of root and shoot biomass allocation on A.senegal and A.sieberiana planted in Soil A and B after being exposed to N fertilizers

Species	Soil substrate	Nitrogen (N) treatment (mg / plant)							
		0		50		100		150	
		Roots (%)	Stems and leaves(%)	Roots(%)	Stems and leaves(%)	Roots (%)	Stems and leaves (%)	Roots (%)	Stems and leaves (%)
A.senegal	A	17.1	82.9	26.3	73.7	19.3	80.7	31.4	68.6
	B	30.0	70.0	29.8	70.2	42.4	57.6	43.2	56.8
A.siberiana	A	23.8	76.2	37.6	62.4	25.7	74.3	32.2	67.8
	B	48.3	51.3	36.9	63.1	41.7	58.3	50.0	50.0

A. senegal planted in unfertilized (N0 treatments) soils B produced higher RGR_{SLB} (0.034 g g^{-1} d^{-1}) compared to those treated with N fertilizer. In the same untreated experiments, it then followed by *A. sieberiana* with RGR_{SLB} of 0.033 g g^{-1} d^{-1} in soil A (Figure 3.2a).

In the experiment treated with N fertilizer, *A. sieberiana* produced higher RGR_{SLB} (0.02 g g^{-1} d-^{1}) in soil B treated with N100 mg / plant than other acacia seedlings in soil A and B treated with N50 and N150 mg / plant (Figure 3.2a). The mean relative root biomass growth (RGR_{RB}) of the two acacia species also varied depending on the level of ammonium nitrate and soil substrate. *A. senegal* planted in unfertilised soil B had higher RGR_{RB} (0.047 g g^{-1} d^{-1}) than those in the fertilised soils (Figure 3.2b).

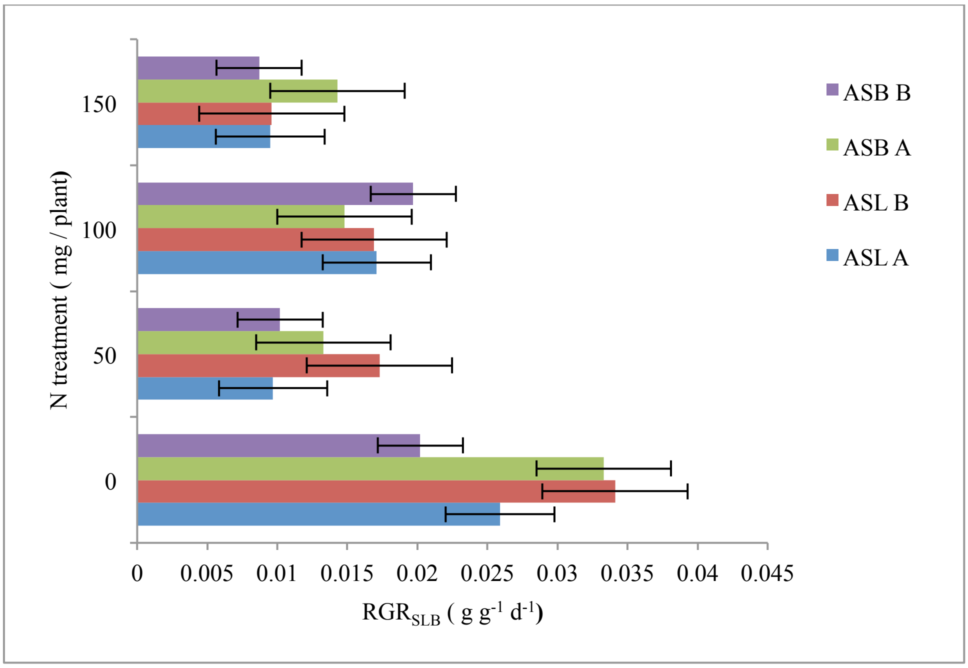


Figure 3.2a. The influence of nitrogen (N) fertilizer on mean relative stem and leaf biomass growth (RGR_{SLB}) of A.senegal (ASL) and A.sieberiana (ASB) seedlings in soil A and B. Bar errors show standard errors.

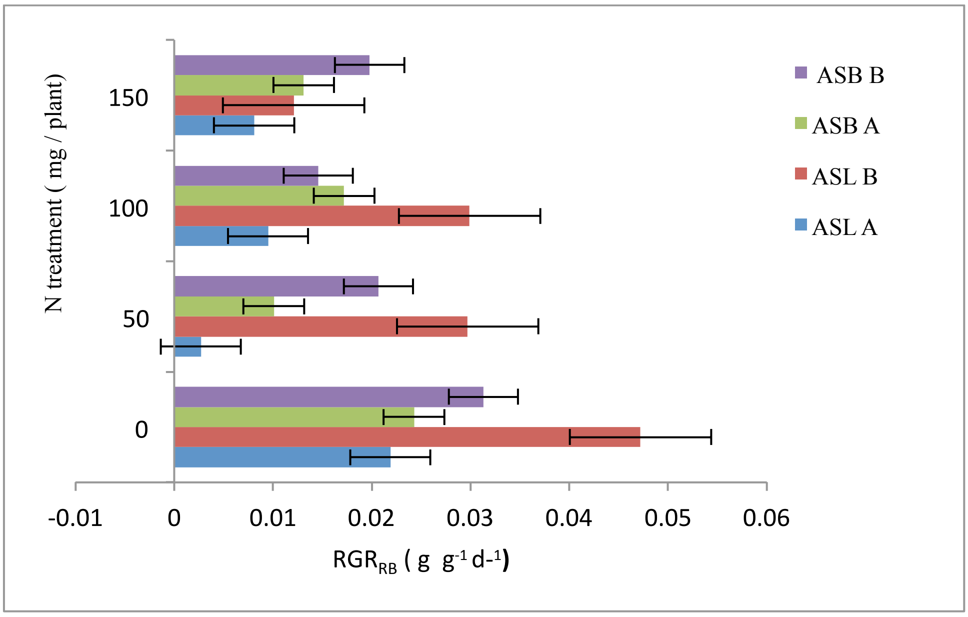


Figure 3.2b. The influence of nitrogen N fertilizer on mean Relative Root Biomass (RGR_{RB}) of Acacia senegal (ASL) and A.sieberiana (ASB) seedlings in soil A and B. Bar errors show standard error.

3.3 Leaf N concentration and Growth traits

The mean leaf N concentration of *A. senegal* and *A. sieberiana* in this study varied depending on the soil substrates and levels of the N treatments (Figure 3.2c). At no fertilization, *A. senegal* seedlings had higher leaf N concentration than *A. sieberiana* (Fig. 3.2c). The seedlings of *A. senegal* in the soil substrates A and B treated with N fertilizers had higher leaf N concentration compared to those in soils with N0 treatments (Figure 3.2c). In all three N treatments (N50, N100 and N150), *A. senegal* had a higher leaf N concentration (7.1%, 6.6%, 6.3%) in soil B respectively compared to the N0 treatments.

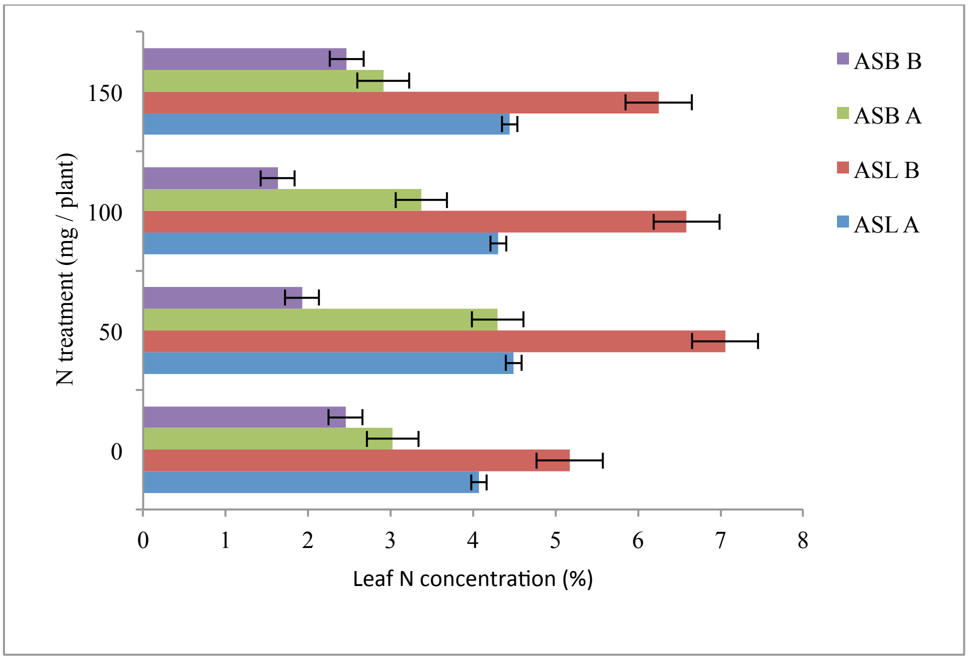


Figure 3.2c. The influence of nitrogen (N) fertilizer on mean leaf N concentration of A.senegal (ASL) and A.sieberiana (ASB) seedlings in soil A and B. Bar errors show standard errors.

On the other hand, *A. sieberiana* had a lower leaf N concentration (1.9%, 1.6%) in soil B treated with N50 and N100 respectively over the N0 treatments (Figure 3.2c).

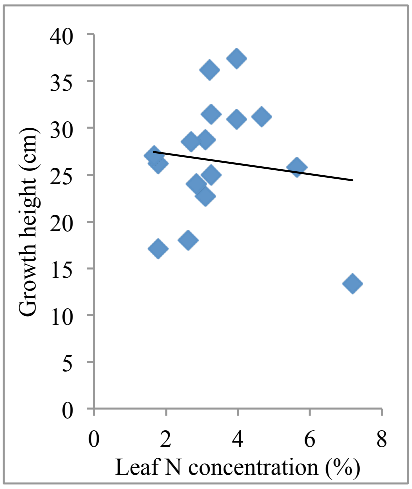
Analysis of correlation of data on the growth traits indicated that there was no significant effect (P≤0.05) of the leaf N concentration on the growth height and leaf length of the two acacia species in all treatments (Table 4).

Table 4. Correlation Analysis between the leaf N concentration and the growth height and leaf length of two acacia species in soil A and B treated with N0, N50,N100 and N150 mg / plant after final harvest

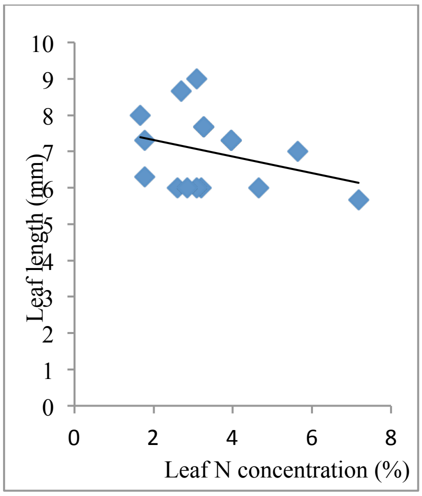
Dependent variable	N0		N50		N100		N150	
	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value
Growth height	-0.12076	0.6558	-0.0638	0.8086	-0.1241	0.6472	-0.4282	0.0980
Leaf length	-0.3169	0.2317	-0.07963	0.7694	-0.0004	0.9988	-0.1966	0..4655

Significant at 0.05

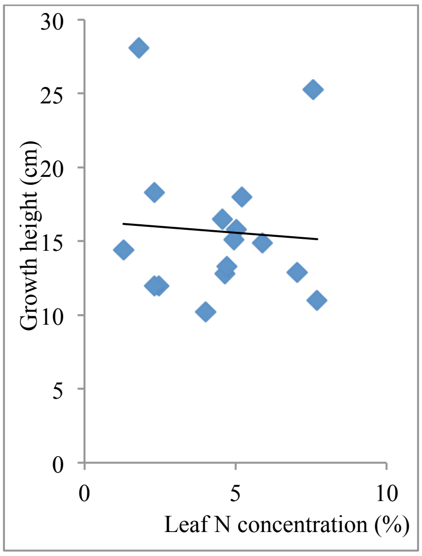
There was a negative correlation between the leaf N concentration and growth traits, i.e growth height and leaf length of the two acacia species in all treatments (Table 4, Figures 3.2 d (i, ii, iii, iv, v, vi,vii,viii)). The growth height of the two acacia species had a higher negative correlation coefficient (-0.43) in soil A and B treated with N150 mg / plant after final harvest compared to other treatments (Table 4, Figure 3.2(vii)). However, the leaf length of the two acacia species had a lower negative correlation coefficient (-0.0004) in soil A and B treated with N100 compared to other treatments (Table 4, Figure 3,2d (vi)).



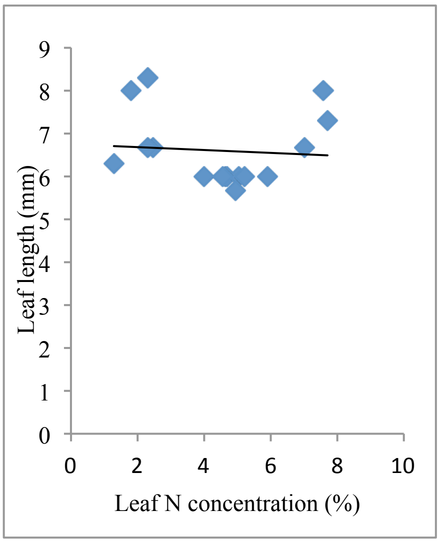
(i)



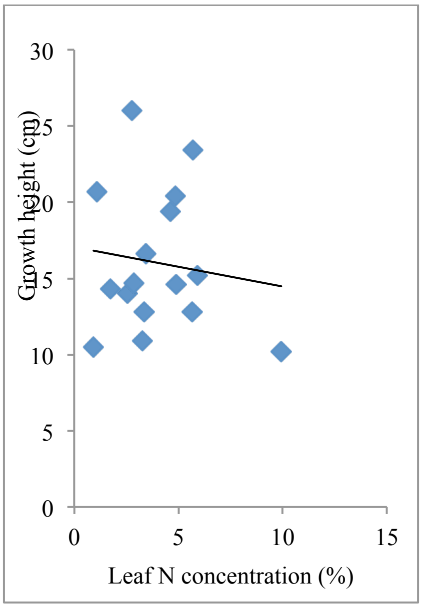
(ii)



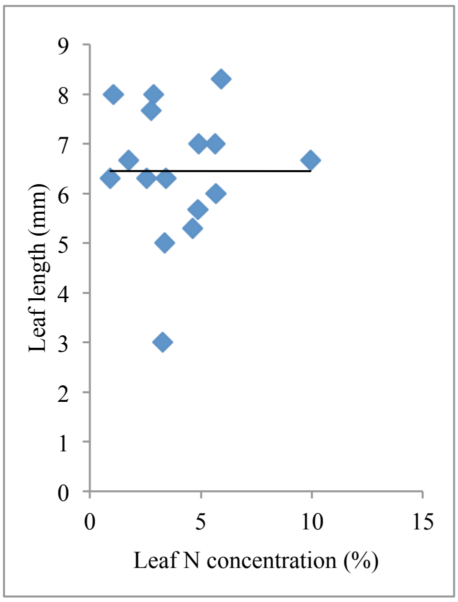
(iii)



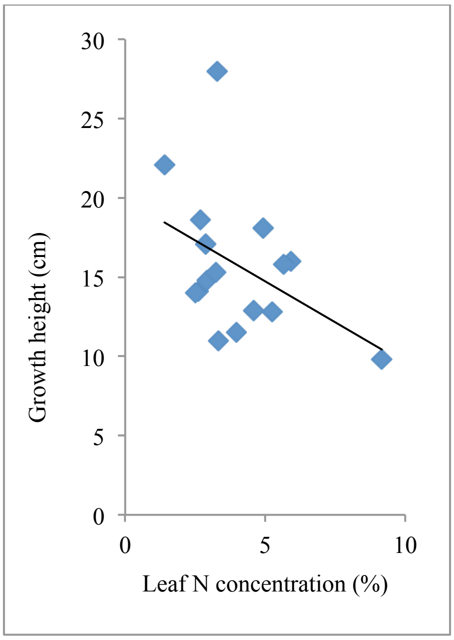
(iv)

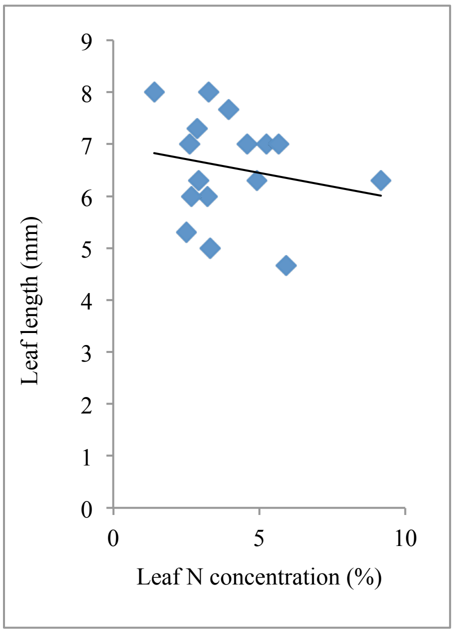


(v)



(vi)





(vii) (viii)

Figure 3.3 d. The influence of the leaf N concentration on the (i) growth heights of the two acacia species in soil A and B treated with N0 after final, (ii) leaf length of two acacia species in soil A and B treated with N0 after final harvest, (iii) growth heights of two acacia in soil A and B treated with N50 after final harvest, (iv) the leaf length of two acacia species in soil A and B treated with N50 after final harvest, (v) growth height of two acacia species in soil A and B treated N100 after final harvest, (vi) leaf length of two acacia species in soil A and B treated with N100 after final harvest, (vii) the growth height of two acacia species in soil A and B treated with N150 after final harvest, (viii) on leaf length of two acacia species in soil A and B treated with N150 after final harvest.

4.0 Discussion

4.1 Growth rate

The results in this study show that there was a general decline in the growth rate (height growth and leaf length) of *A. senegal* and *A. sieberiana* in the experiments treated with N fertilizer compared to those in the unfertilized soils A and B (Figure 3.1a and Figure 3.1b). This suggests that the growth conditions for these two acacia species were altered by the treatment with single N fertilizer doses. In the experiments of this study, addition of large quantities of single nutrients, i.e nitrogen without the proportional addition of other nutrients could have resulted in very strong growth limitations by other nutrients (most likely e.g. phosphorus). The addition of large quantities of ammonium nitrate applied as a salt solution might have exposed these plants to a “salt chock” and hampered their growth. Consequently, there was a very poor growth of all plants exposed to any of the N treatments, except the unfertilised treatments (N0), which did not receive any additional nitrogen (Figure 3.1b).

The results confirm that nutrients need to be added proportional if they are to increase plant growth in nutrient-limited systems (Evans and Edwards, n.d). In addition, the results agree with other findings of Fernandez- Escobar *et al.* (2006) and Boussadia *et al.* (2010) that application of N in excess to the plants does not increase yield or vegetative growth but negatively affect their derived products. This may also cause underground contamination if leached with excess irrigation or rainfall (Alva and Paramasivam, 1998; He *et al*., 2000; Alva *et al*., 2006).

Other studies report that fertilizer salts usually build up when plants are irrigated and affect plants indirectly by changing soil permeability, water and nutrient availability, and directly by ion toxicity (Landis *et al*., 1989; Jacobs and Timmer, 2005; Mindy *et al*., 2008). According to several authors (Van der Mozel et al., 1991; Heth and Macrae, 1993; Nabil and Condret, 1995; Fung et al., 1998; Mehari, 2005), salt tolerance of acacias is affected by the genetics and seed sources of the materials used since plant tolerance to salinity is controlled by genetics. Sands (1981) reports that seeds from non-saline environments also usually show poor germination and seedling growth when they are exposed to salinity. Therefore, it is important to critically evaluate impacts of fertilizers on media chemistry and plant growth before adapting this approach in operational container nursery production of tree seedlings.

Nevertheless, high doses of N fertilization may be important in building up internal nutrient reserves of seedlings by inducing luxury nutrient consumption (Xu and Timmer, 1998). The higher reserves are a readily available source of nutrients for remobilization and retranslocation to new growth soon after planting, a critical period of plantation establishment. The partly very high leaf N concentration in the seedlings exposed to N fertilization in this study indicates some evidence for luxury consumption in those seedlings. Nutritional stress during seedling establishment would be characterized by limited root development in the soil and increased exploitation of internal nutrient reserves (Burdett et al., 1984; van den Driessche, 1985; Burdett, 1990; Xu and Timmer, 1998).

Analysis of variance results show that there was a significant effect of soil substrate on RGR height growth, as well a significant species effect and species x soil interaction on RGR leaf length (Table 2). This suggests that height growth was significantly greater in soil A compared to soil B, regarding leaf length growth: *A. sieberiana* had a much greater leaf length growth than *A. senegal*, and leaf length growth of *A. sieberiana* was lower in soil B compared to soil A, whereas in *A. senega*l, leaf length growth was greater in soil B compared to soil A. This supports the hypothesis that the magnitude of effect of soil substrates on growth rate varies between two acacia species.

Soil A was more porous and lighter than soil B, possibly indicating some differences between the soil substrates used here. Lighter soil possibly facilitates atmospheric N fixation by microorganisms that frequently are associated with acacia roots. Soil A being lighter than soil B could also have allowed water to more easily infiltrate in it, thereby enhancing the growth of the two acacia species.

4.2 Biomass allocation above-ground and below- ground

The results indicate that more biomass was allocated to stems and leaves of *A.senegal* and *A. sieberiana* seedlings compared to their root systems (Table 3). This suggests that the nitrogen supplied by the N and N0 treatments was transported direct to the shoot system of acacia species. In this case, these results are in agreement with the findings that stimulation of leaf growth by N may be greater in those species that transport most of their N directly to the shoot, where accumulation can be used as part of the osmotic force driving cell expansion (Sprent and Thomas, 1984). Nitrogen has been shown to stimulate leaf growth in broad-leaved plants by affecting the rate, rather than the duration of expansion (Armstrong *et al.,* 1986), possibly due to an increase in hydraulic conductivity (Radin & Boyer, 1982). Many plants can accumulate high concentrations of amino acids in their vegetative tissues (Pate, 1983) as a result of luxury consumption of N recovered as soluble proteins, thereby expanding their cells and as well as increasing above-ground biomass.

The results also indicate that *A. senegal* seedlings planted in soil A with N0 treatments had a higher stem and leaf biomass allocation over those of N treatments (Table 3). Similarly, the mean relative stem and leaf biomass growth (RGR_{SLB}) and mean relative root biomass (RGR_{RB}) of *A. senegal* were higher in soil B with N0 treatments compared to those treated with N fertilizers in this study (Figure 3.2a and 3.2b). During the final harvest of the seedlings, it was observed that there was a slight damage on their root tips resulting from the effect of high amount of the N fertilizers applied. Usually, restricted nitrogen or phosphorus availability increases root mass per unit plant mass for monocots, dicots, C4 and woody plants (Cook & Evans, 1983; Cromer & Jarvis.1990; Kirschbaum, Bellingham & Cromer, 1992; Sage & Pearcy, 1987; Evans and Edwards, n.d). In this case, woody plants and forest tree seedlings in particular are sensitive to high electrical conductivity (ECe) levels after N fertilisation and a root damage can easily occur quickly (Landis *et al.,* 1989; Maynard *et al*., 1997), with ECe values as low as 1.4 dS m^{-1} potentially causing growth reduction (Landis, 1988) as discussed above.

Several similar studies also show that N fertilization alters rhizosphere pH by changing relative concentration of different ions in the soil solution (Baligar *et al*. 1998; Bernstein and Kafkafi, 2002). Thus, N is available to plants in both cationic (NH_4^+) and anionic (NO_3^-) forms. Uptake of primarily NO_3^- promotes excess uptake of anions over cations (and release of OH^- from the root) and a subsequent rise in rhizosphere pH, while uptake of NH_4^+ leads to extrusion of H^+ and soil acidification (Neumann and Romheld, 2001). As the pH changes, the availability of these ions as well changes for example P, chemically bound and Fe and Al, easily available. Consequently, this will affect root system development, as high concentrations of Al, for example, tend to be toxic to the roots. This may kill elongating root apical meristems via desiccation and thereby limit root system expansion (Bernstein and Kafkafi, 2002), resulting in decreased root length and dieback of laterals (Baligar *et al*., 1998). Root apical meristems are anatomically suited to act as critical points of nutrient and water uptake (Peterson *et al*., 1999). Thus, an inhibitory response at these sites may negatively affect root system function in addition to retarding root growth, and ultimately lead to limitations in whole-plant development. Therefore, plants top dressed with ammonium nitrate fertilizer will have reduced root length, specific root length (root length per unit of dry matter), and number of root tips (Teng and Timmer, 1995). This suggests that such plants will as well as have reduced root, stem and leaf biomass growth.

The results on ANOVA indicate that there were no significant effects of species, soil substrates and interaction on relative stem and leaf biomass growth (RGR_{SLB}), relative root biomass growth (RGR_{RB}) and root biomass allocation and stem and leaf biomass allocation (Table 2). This does not support the hypothesis that the magnitude of effects of soil substrates on biomass allocation varies between the two acacia species.

On the other hand, there was a significant effect of only the species on the leaf N concentration (Table 2), thereby supporting the hypothesis that the magnitude of soil substrate effects on the leaf N concentration varies between the two acacia species. Therefore, there was no effect on amount of the available soil nutrients on the leaf length and biomass of the two acacia species in 2 months experiment.

4.3 Leaf N concentration and Growth traits

In this study, the results on the leaf N concentration of *A. senegal* in soil types A and B were generally higher in the experiments treated with N fertilizers compared to those with N0 treatments (Figure 3.1c). This suggests that supply of nitrogen to the soil substrates increased its concentration in the leaves of *Acacia* seedlings although it did not lead to increased growth rates. This is also supported by the facts that there was negative correlation between the leaf N and the growth traits of the two acacia species (Table 4, Figures 3.2 d (i, ii, iii, iv, v, vi, vii, viii)), which indicates that additional N reserves were stored in leaves ("luxury consumption") rather than used for growth, because growth was limited by other factors (cf. Other nutrient elements). This further suggests that the seedlings were unable to translate the partly high leaf N concentrations into the growth in this study. These results are not consistent with other findings from studies using more balanced nutrient additions and indicating that the effects of the concentration of N on photosynthesis of leaves may influence growth and partitioning of dry matter in trees (Alerts *et al*., 1992; Malcolm and Ibrahim, 1993; Misra *et al*., 1998), thereby contributing to the increase in the leaf length as well as the plant height.

The results also showed that there was no statistically significant effect of the leaf N concentration on the growth traits of the two acacia species in all treatments (Table 4). This shows that the hypothesis that the leaf N concentration functionally relates to the growth traits of the two acacia species was rejected. Therefore, there was no impact caused the nutrient supply from both soil substrates and the N fertilizers on the growth rate of the two acacia species.

5.0 Conclusions and Recommendations

From the results of this study, it can be concluded that the four N treatments applied to seedlings of *A. senegal* and *A. sieberiana* to determine the effects of their growth rate and biomass allocation varies depending on the parameters.

Unfertilized soil substrates increased the growth heights and leaf length of both *A. senegal* and *A. sieberiana seedlings* more than the N fertilizer treatments. This suggests that amount of N fertilizer applied in this study may not be suitable for biomass production of these acacia seedlings especially at the age of 2 months. Nevertheless, this needs further investigations on a complete and balanced nutrient solution with small quantities of N fertilizers less than the rates used in this study. The age of acacia seedlings can as well as be taken into consideration under nursery conditions.

The results on biomass allocation show that more biomass was allocated to stems and leaves of two acacia seedlings compared to their root system in both N0 and N treatments. However, application of the N treatment produced less stems and leaves biomass allocation compared to N0 treatments. Therefore, N fertilizers may not be used to enhance biomass production of the two acacia species at the age 2 months. Further research can be conducted on the effects of N fertilizer on two acacia species a longer period of experiment.

The two soil substrates affected the growth traits, i.e relative height growth and relative leaf length of the two acacia species differently, thereby supporting the hypothesis that the magnitude of effect of the soil substrates on growth rate varies between the two acacia species. On other hand, the two soil substrates affected the biomass allocation, i.e relative stem and leaf biomass growth, relative root biomass growth, the root biomass allocation and stem and leaf allocation of the two acacia species in the same way, thereby not supporting the hypothesis that magnitude of the effect of the soil substrates on biomass allocation varies between the two acacia species.

There was negative correlation between the leaf N concentration and growth traits of the two acacia species in all treatments. The leaf N concentration also caused no impact on the growth traits of the two acacia species in all treatment. Therefore, the hypothesis that the leaf N concentration functionally relates to the growth of the two acacia species was not supported.

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